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# Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* Strains in Various Types of Private Health Care Centers▽

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**During a 2004 survey, 49 extended-spectrum-β-lactamase-producing enterobacteria were collected in 20 French private health care centers and one local hospital. They included 12 CTX-M-producing *Escherichia coli* strains (1.8% versus 0.3% in a 1999 survey). Most of them belonged to the same clone and contained a $bla_{CTX-M-15}$ gene on similar conjugative plasmids.**

Extended-spectrum β-lactamases (ESBLs) are one of the most significant mechanisms of resistance to oxyiminocephalosporin antibiotics in *Enterobacteriaceae* (6). In the 1980s, the ESBLs were predominantly TEM and SHV derivatives (6). However, since 2000, the CTX-M enzymes, originally described in South America, Asia, and Eastern Europe, have spread worldwide (4). In parallel, nosocomial outbreaks due to isolates expressing plasmid-mediated class C enzymes have been increasingly reported (18, 21).

In France, antibiotic resistance is regularly monitored in the hospital environment (20, 28) but is less well documented in private health care centers, which can be surveyed only by private laboratories in charge of their microbiological tests (23). In 1998, we founded a network of private laboratories to monitor antibiotic resistance in the extrahospital practices of the Aquitaine region (in southwestern France). In 1999, a survey of the Aquitaine network revealed that ESBL-producing enterobacteria were present in private health care centers at rates similar to those found in hospitals (23) and were occasionally responsible for authentic outbreaks in these facilities (2). The aim of this study was to perform a similar survey focused on private health care centers in order to analyze the incidence of ESBL-producing enterobacteria according to the type of institution and to examine the evolution over the five preceding years.

From January to June 2004, 1,570 clinically relevant, consecutive, and nonredundant strains of enterobacteria were collected from institutionalized patients by the Aquitaine network. ESBLs were detected by the double-disk synergy test (13) with or without the presence of cloxacillin, which inhibits cephalosporinases (26). ESBLs were found in 49 strains, mainly isolated from the urinary tract (41 samples) of 45 institutionalized patients (total, 1,398 patients) residing in 21 of the 69 examined centers (7/19 clinics; 4/8 "follow-up" centers, including 2/6 convalescence and 2/2 rehabilitation centers, 9/40 nursing homes, and 1/2 local hospitals). The global proportion of private institutions accommodating patients infected with ESBL-positive enterobacteria (omitting the local hospitals absent from the 1999 survey) was similar (29.4% versus 29.8% in 1999; $P < 0.005$). The overall incidence of ESBL producers in private health care centers slightly decreased compared to 1999 (2.8% versus 3.3%), but not significantly ($P = 0.51$). The variations observed between the two surveys can be ascribed, at least in part, to the evolution of the network (16 laboratories in 2004 versus 8 in 1999). The percentages of ESBL producers in private facilities increased from the clinics (1.5%) and convalescence centers (1.7%) to rehabilitation centers (5.3%) and nursing homes (10.9%). This rank order correlates with a longer stay (e.g., mean duration, 7 days in clinics versus 58 days in rehabilitation centers). Nursing homes are known to be an important reservoir of ESBL-producing enterobacteria (19). The high frequency of ESBL producers in local hospitals (11.1%) shows that these public institutions, too small to have their own laboratories and excluded from public statistics, can be heavily contaminated. In addition, plasmid-mediated cephalosporinases were found in two strains recovered from the urinary samples of two patients residing in CLI-2/RC-1, one of which also expressed an ESBL (Table 1). Thus, a total of 50 strains were analyzed, including 49 ESBL-expressing isolates and one plasmidic AmpC-expressing isolate.

Identification to the species level by the API20E system (bioMérieux, Marcy-l'Etoile, France) and molecular tests showed that the 50 strains comprised 18 *Enterobacter aerogenes*, 7 *Klebsiella pneumoniae* (6 with ESBL and 1 with a

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TABLE 1. Characteristics of the enterobacteria expressing ESBLs and/or plasmid-mediated cephalosporinases

| Species (total no. of strains) | No. of ESBL/ cephalosporinase-positive strains (%) | Molecular type[a,b] | Location[b,c] | Specimen[b] | β-Lactamase content and antibiotype[d] |
|---|---|---|---|---|---|
| *E. aerogenes* (53) | 18 (34.0) | Ea1 (5) | CLI-1 (2), H-1 (2), NH-6 | Urine (3) Respiratory (2) | **TEM-24b** AmpC **TNt(A) SSS TMP C** OFX |
| | | Ea1 (5) | CLI-2/RC-1(4), CLI-10 | Urine (5) | **TEM-24b TNt(A) SSS TMP C** OFX |
| | | Ea1 (2) | CLI-12/CC-1 (2) | Urine (2) | **TEM-24b** AmpC **(T) SSS C** OFX |
| | | Ea1 (2) | CLI-5, NH-10 | Urine (2) | **TEM-24b** AmpC **(T) SSS TMP C** OFX |
| | | Ea1 | NH-6 | Urine | **TEM-24b** AmpC **TEM-2 TNt(A) SSS TMP C** OFX |
| | | Ea1 | CLI-2/RC-1 | Urine | TEM-24b AmpC TEM-1 TNt(A) SSS TMP C OFX |
| | | Ea2 | NH-8 | Urine | **TEM-3** AmpC **TNt(A) SSS TMP C** OFX |
| | | Ea2 | CLI-5 | Pus | **TEM-3 TNt(A) SSS TMP C** OFX |
| *E. coli* (933) | 17 (1.8) | Ec1 (4) | H-1 (2), NH-5 (2) | Urine (4) | CTX-M-15 TEM-1 OXA-1 GTNt(A) TE OFX |
| | | Ec1 | H-1 | Urine | **CTX-M-15 TEM-1 OXA-1 GTNt(A) TE** OFX |
| | | Ec2 | CLI-13 | Urine | CTX-M-15 TEM-1 OXA-1 TNt(A) SSS TMP TE OFX |
| | | Ec2-a | NH-11 | Urine | CTX-M-15 OXA-1 TNt(A) SSS TMP OFX |
| | | Ec2 | CLI-13 | Urine | CTX-M-15 TEM-1 OXA-1 TNt(A) TE OFX |
| | | Ec3 | RC2 | Urine | CTX-M-15 TEM-1 OXA-1 TNt(A) TE OFX |
| | | Ec4 | CLI-15 | Urine | **CTX-M-1** AmpC SSS TMP TE OFX |
| | | Ec5 | CLI-4 | Urine | **CTX-M-2 TEM-1 SSS TMP** C **TE** NAL |
| | | Ec6 | NH-9 | Urine | **CTX-M-14** C TE OFX |
| | | Ec7 (2) | NH-7 (2) | Urine (2) | SHV-12 IRT-6 GTNt(A) OFX |
| | | Ec8 | NH-1 | Urine | **TEM-21** AmpC **GTNt(A) SSS** TMP **C** OFX |
| | | Ec9 | NH-4 | Urine | **TEM-24b TEM-26 TNt(A) SSS TMP TE** |
| | | Ec10 | CC-2 | Pus | **TEM-24b TNt(A) SSS TMP C** OFX |
| *K. pneumoniae* (104) | 7 (6.7) | Kp1 | CLI-1 | Respiratory | SHV-4 SHV-1c TNt(A) SSS TMP C OFX |
| | | Kp2 | CLI-5 | Urine | TEM-15 SHV-1c SSS TMP NAL |
| | | Kp2 | CLI-14 | Blood | TEM-15 SHV-1c SSS TMP NAL |
| | | Kp2 | NH-8 | Urine | **TEM-15 TEM-17** TEM-1 SHV-1c GTNt(A) SSS TMP OFX |
| | | Kp3 | CLI-1 | Urine | **SHV-2a TEM-1** SHV-1c **GTNt** NAL |
| | | Kp4 | CLI-2/RC-1 | Urine | **SHV-12 SHV-1 CMY-4**[d] SHV-1c **GTNt(A) SSS TMP C** OFX |
| | | Kp5 | CLI-2/RC-1 | Urine | ACC-I[e] TEM-1 SHV1c SSS TMP C OFX |
| *P. mirabilis* (153) | 4 (2.6) | Pm1 | CLI-2/RC-1 | Pus | **TEM-24b TNt(A) SSS** TMP **C** TE |
| | | Pm2 | RC-2 | Urine | **TEM-24b TNt(A) SSS TMP C** TE |
| | | Pm3 | NH-4 | Urine | **TEM-24b TNt(A) SSS TMP** TE OFX |
| | | Pm4 | NH-1 | Urine | **TEM-21 GTNt(A) SSS** TMP **C** TE OFX |
| *E. cloacae* (87) | 2 (2.3) | Ecl 1 (2) | CLI-1 | Urine, pus | **TEM-24b** AmpC **GTNt(A) SSS TMP C** OFX |
| *M. morganii* (60) | 1 (1.7) | ND[f] | H-1 | Urine | **TEM-24b** AmpC **SSS TMP C** TE OFX |
| *K. oxytoca* (60) | 1 (1.7) | ND | CLI-1 | Urine | **TEM-11 GTNt(A) SSS TMP C** TE OFX |

[a] A single number assigned according to the concordant results obtained by all molecular typing methods.
[b] The number of isolates is given in parentheses if more than one isolate was recovered.
[c] CLI, clinic; NH, nursing home; H, local hospital; RC, rehabilitation center, CC, convalescence center. CLI-1 to CLI-12 and NH-1 are the institutions described by Arpin et al. (2, 3).
[d] The cotransferred resistances with ESBLs are indicated in boldface. G, K, T, Nt, and A represent gentamicin, kanamycin, tobramycin, netilmicin, and amikacin, respectively; C, chloramphenicol; SSS, sulfamethoxazole; TMP, trimethoprim; TE, tetracycline; NAL, nalidixic acid; OFX, ofloxacin. Parentheses indicate a low level of resistance. AmpC and SHV-1c are species-specific cephalosporinase and penicillinase, respectively.
[e] Plasmid-mediated cephalosporinase.
[f] ND, not determined.

cephalosporinase), 17 *Escherichia coli*, 4 *Proteus mirabilis*, 2 *Enterobacter cloacae*, 1 *Klebsiella oxytoca*, and 1 *Morganella morganii* strain (Table 1). Thus, while the proportion of ESBL-producing strains remained stable within the *E. aerogenes* species (33.0% of this species versus 32.0% in 1999), it drastically increased within the *E. coli* species (1.8 versus 0.3%). Among the ESBL producers, the *E. aerogenes* strains were less predominant than in 1999 (32.6% versus 47.0%), while the importance of the *E. coli* strains grew (34.7% versus 14.7%), as reported in French University hospitals (15).

ESBL producers were multidrug resistant, as shown by the agar diffusion method using 27 disks (27) (Table 1). Indeed, most of the 50 strains were resistant to aminoglycosides, including 24 that exhibited a TNt(A) phenotype (tobramycin, netilmicin, and low-level amikacin resistance) associated with an *aac(6′)-I* gene, 1 strain with a GTNt phenotype (gentamicin, tobramycin, and netilmicin resistance) related to an *aac(3)-II* gene, and 12 strains with the combined GTNtA phenotype and both enzyme-encoding genes, as verified by PCR amplifications (2, 3, 8, 9). The strains also exhibited resistances to sulfonamides (78%), trimethoprim (74%), chloramphenicol (64%), and tetracycline (37%) (Table 1). Although most ESBL-positive strains were resistant to nalidixic acid (94%) and ofloxacin (86%), no *qnr* genes could be found using a previously published method (12, 24). However, eight *E. coli* produced the *aac(6′)-Ib-cr* variant, as demonstrated after gene sequencing. Only carbapenems were active against all 50 strains (Table 2).

TABLE 2. β-Lactam susceptibilities of strains

| β-Lactamase content (molecular type and CTX-M-15-associated plasmid profile) | No. of strains | MIC (μg/ml)[a]: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | AMX + CA | FOX | CTX | CTX + CA | CAZ | CAZ + CA | IPM | ERT |
| *E. aerogenes* (18 strains) | | | | | | | | | |
| TEM-24b AmpC ± TEM-1 or TEM-2 (Ea1) | 11 | 32–>512 | 512–>512 | 1–8 | 1–8 | 256–512 | 4–2 | 0.5–2 | 0.05–0.2 |
| TEM-24b (Ea1) | 5 | 4–64 | 8–32 | 2–1 | 0.1–0.2 | 128–512 | 1–4 | 0.5–1 | 0.02 |
| TEM-3 AmpC (Ea2) | 1 | 128 | 128 | 4 | 0.1 | 16 | 1 | 1 | 0.2 |
| TEM-3 (Ea2) | 1 | 8 | 32 | 0.2 | 0.1 | 16 | 0.5 | 0.5 | 0.02 |
| *E. coli* (17 strains) | | | | | | | | | |
| CTX-M-15 TEM-1 OXA-1 (Ec1, pEc1-A) | 5 | 32–64 | 4–8 | 256 | 0.1 | 64 | 0.2 | 0.5 | 0.05 |
| CTX-M-15 TEM-1 OXA-1 (Ec2, pEc1-B) | 1 | 128 | 8 | 64 | 0.1 | 16 | 1 | 0.5 | 0.1 |
| CTX-M-15 OXA-1 (Ec2-a, pEc1-C) | 1 | 32 | 8 | 256 | 1 | 256 | 2 | 0.5 | 0.2 |
| CTX-M-15 TEM-1 OXA-1 (Ec2, pEc1-D) | 1 | 256 | 32 | 2 | 0.2 | 1 | 0.5 | 0.5 | 0.05 |
| CTX-M-15 TEM-1 OXA-1 (Ec3) | 1 | 128 | 32 | 512 | 1 | 256 | 4 | 0.5 | 0.1 |
| CTX-M-1 AmpC (Ec4) | 1 | 256 | 64 | 64 | 2 | 8 | 8 | 1 | 0.05 |
| CTX-M-2 TEM-1 (Ec5) | 1 | 8 | 4 | 32 | 4 | 1 | 0.1 | 0.5 | 0.05 |
| CTX-M-14 (Ec6) | 1 | 4 | 4 | 32 | 0.1 | 4 | 1 | 0.5 | 0.02 |
| SHV-12 IRT-6 (Ec7) | 1 | 128 | 64 | 16 | 0.5 | 128 | 1 | 0.5 | 0.2 |
| SHV-12 IRT-6 (Ec7) | 1 | 256 | 64 | 128 | 8 | 512 | 2 | 1 | 0.5 |
| TEM-21 AmpC (Ec8) | 1 | 256 | 128 | 32 | 2 | 64 | 16 | 0.5 | 0.1 |
| TEM-24b TEM-26 (Ec9) | 1 | 8 | 4 | 1 | 0.1 | 128 | 1 | 0.5 | ≤0.01 |
| TEM-24b (Ec10) | 1 | 8 | 8 | 2 | 0.1 | 256 | 1 | 0.5 | 0.02 |
| *K. pneumoniae* (7 strains) | | | | | | | | | |
| SHV-4 (Kp1) | 1 | 4 | 16 | 8 | 0.1 | 128 | 0.5 | 0.5 | 0.02 |
| TEM-15 (Kp2) | 1 | 32 | 8 | 32 | 0.2 | 64 | 2 | 1 | 0.1 |
| TEM-15 (Kp2) | 1 | 8 | 4 | 8 | 0.1 | 32 | 0.5 | 0.5 | 0.02 |
| TEM-15 TEM-17 TEM-1 (Kp2) | 1 | 8 | 64 | 64 | 64 | 512 | 64 | 0.5 | 0.1 |
| SHV-2a TEM-1 (Kp3) | 1 | 8 | 4 | 2 | 0.1 | 256 | 0.5 | 1 | 0.02 |
| SHV-12 SHV-1 CMY-4[b] (Kp4) | 1 | 128 | 32 | 16 | 4 | 4 | 16 | 1 | 0.01 |
| ACC-I[b] TEM-1 (Kp5) | 1 | 128 | 8 | 8 | 8 | 64 | 64 | 0.5 | 0.05 |
| *P. mirabilis* (4 strains) | | | | | | | | | |
| TEM-24b (Pm1 to Pm3) | 3 | 1 | 2–4 | 0.5–1 | 0.01–0.02 | 8–16 | 0.05–0.1 | 1 | 0.01 |
| TEM-21 (Pm4) | 1 | 16 | 4 | 2 | 0.02 | 1 | 0.1 | 1 | 0.01 |
| Other species (4 strains) | | | | | | | | | |
| TEM-24b AmpC TEM-1 (*E. cloacae*, Ecl1) | 2 | >512 | 512 | 64–128 | 64–128 | 512–>512 | 128 | 1 | 0.5–1 |
| TEM-24b AmpC (*M. morganii*) | 1 | >512 | 128 | 16 | 16 | 128 | 32 | 4 | 0.02 |
| TEM-11 (*K. oxytoca*) | 1 | >512 | 8 | 1 | 0.1 | 128 | 1 | 0.5 | 0.1 |

[a] AMX, amoxicillin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem, ERT, ertapenem. Clavulanic acid (CA) was used at a fixed concentration of 2 μg/ml.
[b] Plasmid-mediated cephalosporinase.

The β-lactamase contents of the strains were determined by isofocusing; PCR amplification using $bla_{TEM}$-, $bla_{SHV}$-, and $bla_{CTX\text{-}M}$-specific primers (2, 3); and sequencing. When several enzymes of the same TEM or SHV lineage coexisted, sequencing was performed after separation of the amplicons by cloning (2, 3). Following this procedure, a much greater variety of ESBLs were identified than in 1999, i.e., 14 versus 7 enzymes. In both cases, the ESBLs were divided into TEM-type (67.3% versus 96.1% in 1999), SHV-type (8.2% versus 0%), and CTX-M-type (24.5% versus 2.9%) enzymes, but in strikingly different proportions, highlighting the decline of the conventional ESBLs and the upsurge of the CTX-M family. While TEM-24b and TEM-21, widely predominant in 1999, remained common in 2004, TEM-3, TEM-15, SHV-4 (in the same patient after a 5-year interval) and CTX-M-1 were sporadically identified in both periods. In contrast, TEM-11 (first described in *K. oxytoca*), TEM-17, TEM-26, SHV-2a, and SHV-12 appeared to have emerged in the Aquitaine region.

Besides antibiotyping, the epidemiological relationship between strains belonging to the same species has been investigated by pulsed-field gel electrophoresis using a CHEF-DRIII system (Bio-Rad), and the restriction endonucleases XbaI (*E.*

*coli*, *E. aerogenes*, and *K. pneumoniae*), SpeI (*E. cloacae*), and SfiI (*P. mirabilis*). The obtained patterns were interpreted according to the criteria of Tenover et al. (29) (Table 1). Resistances cotransferred with ESBLs and AmpC enzymes were studied by a filter-mating assay using a nalidixic acid- and rifampin-resistant mutant of *E. coli* K-12 or an azide-resistant *E. coli* C600 as a recipient (2, 3). Restriction plasmid profiles were determined after plasmid DNA extraction (25) by EcoRI or HpaI digestion (Promega, Charbonnière-les-Bains, France).

The combined analysis of β-lactamase content and typing methods demonstrated that among the 18 *E. aerogenes* strains, 16 belonged to the molecular type Ea1 and produced the TEM-24b enzyme. The Ea1 clone was highly similar to that previously epidemic in our region and prevalent in France and bordering countries at the end of the 1990s (1–3). This clone was responsible for an outbreak in CLI-2 in 1999 and still persisted in the clinic in 2004, albeit at a lower rate (4.7% versus 8.7%). However, it had unexpectedly evolved toward increased susceptibility, consistent with a genetic shift associated with its progressive decline. Indeed, not only were none of the Ea1 isolates resistant to imipenem (versus 12% in 1999), but the chromosomal AmpC β-lactamase was not inducible in six of them (28% versus 0% in 1999), leading to atypical amoxicillin-clavulanate and cefoxitin susceptibilities (7) (Table 2). Moreover, four *E. aerogenes* strains showing only a decreased tobramycin susceptibility [(T) phenotype] possessed an *aac(6')-I* gene governed by a weak promoter, as shown by sequencing (data not shown). As reported in 1999, the conjugative plasmid encoding the TEM-24b enzyme was found in eight strains belonging to four other enterobacterial species, and it occasionally underwent limited variations, sometimes associated with the loss or gain of cotransferred resistances (2). Similarly, a highly transferable TEM-21-encoding plasmid, widespread in the Bordeaux area (2, 8, 30) and responsible for an outbreak in NH-1 in 1999, was still present in that institution in 2004 (identical EcoRI patterns) (data not shown). The TEM-3-producing clone of *E. aerogenes* Ea2 and the TEM-15-producing clone of *K. pneumoniae* Kp2 also persisted in the Southern "Pays Basque" part of our region, particularly in CLI-5.

However, one of the major differences from the 1999 survey was the increase in CTX-M-expressing *E. coli* strains (24.5% versus 2.6%). Among the 17 ESBL-producing *E. coli* strains, 12 expressed a CTX-M enzyme, including 9 that elaborated CTX-M-15. Of these nine strains, scattered among five institutions, five exhibited the molecular type Ec1, as did two control strains (TN03 and DOS) collected in northern French hospitals (11, 14). Moreover, the five Ec1 *E. coli* strains showed the same HpaI-restricted plasmid profile (pEc1-A), antibiotic resistance patterns, and β-lactam MICs (Table 1), providing evidence of the dissemination of a clonal strain in the local hospital and a nearby nursing home. The pEc1-A plasmid was identical to pTN03, a plasmid extracted from the control strain, TN03, which carries an IS*Ecp1*-like element upstream from $bla_{\text{CTX-M-15}}$ (10). pTN03 possesses the same $bla_{\text{CTX-M-15}}$ environment as Pc15-1a, an epidemic plasmid originally described in Canada (5). Among the four other CTX-M-15-producing *E. coli* strains, three were Ec2 or Ec2-a types (possibly related, according to the criteria of Tenover et al.), like the MTPB5 control strain from a southwestern French hospital (15). The Ec2/Ec2-a strains presented different HpaI-restricted plasmid patterns, cotransferred resistances, and insertion sites of IS*26* within the IS*Ecp1*-like elements (data not shown). In particular, this insertion in the pEC1-D plasmid generated a weak promoter, translating into low cefoxatime MICs (Table 2). CTX-M-15 is currently the predominant CTX-M enzyme worldwide, and its propagation has previously been attributed to its presence in an epidemic clone of *E. coli* belonging to the phylogenetic group B2, which expresses multiple virulence factors (16, 17, 22). The remaining strains of *E. coli*, including three other CTX-M producers, were unrelated, except for two (profile Ec7) that were isolated from the same patient but were considered nonduplicates because of their different β-lactam susceptibilities. These differences were due to the presence or absence of IS*26*, providing a strong promoter upstream from the $bla_{\text{SHV-12}}$ gene (Tables 1 and 2). Analysis of all collected enterobacteria, including the 184 redundant ones, underscored prolonged ESBL carriage (6 days to 3 months), in vivo interspecific transfer in the same patient, ESBL acquisition by new patients in known contaminated facilities, and interinstitution transfer of contaminated patients.

The other main difference from the 1999 survey was the detection of the two plasmid-mediated cephalosporinase-producing *K. pneumoniae* strains. One strain (Kp4) expressed the CMY-4 enzyme associated with the SHV-12 ESBL, while the other (Kp5) produced the ACC-1 enzyme and exhibited the same molecular pattern as SLK 54, a control strain involved in a nosocomial outbreak in the north of France several years before (18). Our study is the first description of the emergence of plasmid-mediated cephalosporinases in the French extrahospital setting.

In conclusion, almost one-third of French private health care centers accommodated patients infected with ESBL-producing strains. The frequencies of these organisms varied according to the type of private institution and was highest in nursing homes and rehabilitation centers. Compared to a survey 5 years before, ESBL producers were isolated at a globally similar rate but showed a drastic change in species and enzyme distributions. Indeed, the previously epidemic TEM- or SHV-producing *E. aerogenes* or *K. pneumoniae* strains tended to be supplanted by CTX-M-expressing *E. coli* strains. Unusual phenotypes and variable levels of expression of the same ESBL in relation to promoter modifications were observed. Owing to the complex epidemiology of ESBL producers, regular surveys in private health care institutions are warranted.

**Nucleotide sequence accession number.** The partial nucleotide sequence of the *rpoB* gene from *E. aerogenes* strain Ea2822 has been assigned the GenBank/EMBL database accession number EF108305.

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